Characterization of a Partial Sequence Encoding Envelop Protein of HCV-Genotype 4a Egyptian Isolate

Shawky H.1, Maghraby A. S.1, Solliman M.2, El. Mokadem M. T.3, Sherif M. M.4, Arafa A.1, Bahgat M. M.1

1The Immunology and Infectious Diseases Laboratory, Therapeutic Chemistry Department, the Center of Excellence for Advanced Sciences, National Research Centre, Dokki, Giza, 12622, Egypt
2Vaccines Laboratory, Plant Biotechnology Department, Center of Excellence for Advanced Sciences, National Research Centre, Dokki, Giza, 12622, Egypt
3Botany Department, Faculty of Girls, Ain-Shams University
4Microbiology and Immunity Department, Faculty of Medicine, Al-Azhar University
5Research Group of Biomarkers for Infection Research, Institute of Experimental Infection Research, TWINCORE Centre for Experimental and Clinical Infection Research, Feodor-Lynen-Straße 7-9 D - 30625 Hannover

ABSTRACT: In this study, RNA isolated from sera of Egyptian HCV-patients was used to amplify a fragment of a M. wt. of ~800pb corresponding to a partial sequence of the HCV-E2 encoding gene. The amplified fragment was cloned, sequenced and the nucleotide blast analysis of our sequence revealed partial homology with previously published E2-genes of viral isolates from different locations; the highest match (88%) was annotated with a Japanese isolate suggesting that our herein characterized HCV-E2 partial sequence is a novel one. The impact of HCV-E2 sequence variability will be discussed.

I. Introduction

About 150 million people are chronically infected with hepatitis C virus worldwide, and more than 350 000 people die every year from hepatitis C-related liver diseases [1]. Egypt has the highest worldwide prevalence of genotype 4 as the most common in the Middle East and Africa [2]. The HCV genome is a positive-strand, ~ 9.6-kb RNA molecule consisting of a single open reading frame (ORF) flanked by 5′ and 3′ untranslated regions (UTRs). The 5′UTR contains a highly structured internal ribosome entry site while the 3′ UTR is essential for replication [3, 4]. The HCV-ORF encodes a single polyprotein of ~ 3000 amino acids in length and is post translationally processed to produce at least 10 different proteins: the core, envelope proteins (E1-E2), p7 and the non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B [5, 6, 7]. The envelope glycoprotein genes display some of the highest levels of HCV genetic heterogeneity, with E2 exhibiting greater variability than E1. The hypervariable region 1 (HVR1) is located at the N-terminus of E2 and this region is the major determinant for strain-specific neutralizing-antibody responses [8]. The rate and nature of nucleotide substitutions within HVR1 during the early stages of infection appear to be correlated with outcome: patients harboring a stable HVR1 quasispecies frequently resolve infection, whilst those with evidence of a rapidly evolving population develop chronic infection. In spite of the high variability of this region there is a strong negative selection against some amino acid substitutions since, at most codons, there is selective pressure for conservative amino acid substitutions since, at most codons, there is selection for conservative amino acid replacement, pointing to a biological role in the virus life-cycle [9, 10]. E2; one of the possible targets for the development of an effective vaccine, encodes as many as 11 N-linked glycosylation sites, many or all of which may be utilized during the post-translational processing of nascent E1-E2 complexes [11]. Multiple N-linked glycans, in addition to assisting in the folding of antigenically complex proteins, may have other functions, such as masking proteins from reactivity with virus-specific antibodies, facilitating escape from neutralization by antibodies or the complement, and interfering with antigen processing. E2 is thought to mediate attachment to target cells and binds to human CD81, a member of the tetraspanin family of proteins. Interaction of E2 with CD81 on B or T cells has been reported to result in B-cell aggregation and a lowering of the threshold for T- and B-cell activation [12, 13, 14]. The N-terminal 27 residues of E2 (HVR1); aa 384–410, show a very high degree of variation, both within isolates and genotypes, and this portion of the sequence is considered as a leading contributor to disease progression due the emergence of new viral mutants or “quasispecies” induced by the host immune system [15, 16, 17]. This study was designed to amplify HCV-E2 protein encoding sequence from HCV-infected Egyptian patients and compare it to other HCV-envelope sequences from different geographical settings.
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II. Materials and Methods

2.1. Human Sera

Blood samples were collected from infected Egyptian patients with HCV who were examined at the Medical Unit of the National Research Center. Additional blood samples were collected from humans with no history of liver diseases or infections and used as negative control. Both were centrifuged at 14,000xg, sera were separated, divided into aliquots and stored at -80 °C until used for viral RNA extraction, measuring liver enzymes and detecting of anti-HCV antibodies (Axium; Florida, USA).

2.2. Approval of the Ethical Committee

Collection of human blood samples was approved by the Medical Ethical Committee of the National Research Center in Egypt according to the ethical guidelines approved by the Ethical Committee of the Federal Legislation and ethical guidelines of the National Institutes of Health in the USA.

2.3. Amplification of HCV-E2 Gene

Viral RNA was extracted from sera of HCV infected patients according to the manufacturer’s instructions, (Qiagen, Hilden, Germany). The HCV-E2 encoding sequence was amplified using the listed primers in (Table 1) in either a single round or nested reverse transcriptase polymerase chain reaction (RT-PCR) and the RevertAid premium RT (Thermofisher Scientific; USA). The RT program included 60 min at 50 °C followed by enzyme deactivation at 85°C for 5 min. The first PCR program included 35 cycles each of 30 seconds (s) at 94°C, 45 seconds at 54°C and 2 min at 72°C using a DreamTag DNA polymerase (Thermofisher Scientific, USA), followed by a final extension at 72 °C for 10 min. The PCR product was used in the nested PCR and the program included 35 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C and a final extension of 72 °C for 10 min. PCR products were resolved by electrophoresis on 1% agarose gels, gel slices carrying the amplified fragments were cut and subjected for DNA extraction (Gel cleaning kit; Thermofisher Scientific, USA).

2.4. Cloning, Plasmid Purification and Sequencing

The purified envelope fragment was first cloned into the pSC-TA plasmid (Stratagen) according to the user manual and the generated construct (pSC-E2) was used to transform the DH5α E. coli competent cells. Successful cloning was confirmed by colony PCR on grown bacteria on LB/ampicillin agar plates. PCR-positive colonies were subjected to small-scale plasmid preparation using the GeneJet plasmid DNA miniprep kit (Thermofisher Scientific, USA), the purified plasmid was subjected to automated sequencing from both directions using the HCV-E2 sequence specific forward (F) and reverse (R) primers. The obtained sequences were aligned to the previously published sequences in the GenBank using the basic nucleotide blast analysis [18].

III. Results & Discussion

3.1. Successful Amplification and Cloning of the Encoding Fragment for the HCV-E2 Protein

A fragment of a M. wt. of ~800pb corresponding to a partial sequence of the HCV-E2 encoding gene was visualized upon subjecting extracted RNA from HCV-infected human sera to RT-PCR (Fig. 1.A). Successful amplification of HCV-E2 fragment was first confirmed by internal PCR that resulted in a shorter fragment of 660pb when as expected molecular weight predicted from the published sequence of HCV.Ed43 (Accession NO. Y11604) (Fig. 1.B). Moreover, successful cloning to pSC-TA vector was verified when the same parent fragment (800 bp) was detected by direct PCR on transformed bacteria with the construct carrying such a fragment (Fig 2).

3.2. Homology of the Obtained Sequence with Previously Published HCV-E2 Gene Sequences from Various Geographical Settings

Nucleotide blast analysis (ncbi.nlm.nih.gov) of our HCV-E2 partial sequence revealed partial homology with previously published E2-genes of viral isolates from different locations such as Egypt, Japan, USA, UK and others .The highest extent of homology (88%) was annotated with the sequence of hepatitis C virus subtype 4a genomic RNA, complete genome, isolate: HCV genotype 4a-KM (AB795432.1, Tsukiyama-Kohara, K. and Michinori Kohara, 2013 (unpublished)), which further confirms the successful amplification of the HCV-E2 fragment and strongly suggest a correlation between such sequence variation and the resistance of Egyptian HCV 4a –infected patients to any HCV therapy including the novel ones containing the protease inhibitors [18]. The descending order of the homology of our HCV-E2 partial nucleotide sequence with previously published ones is presented in Table (2). Nucleotide sequence-based phylogenetic tree is demonstrated in (Fig. 3).
IV. Conclusion

Both partial homology and unique features of our newly characterized HCV-E2 sequence compared to previously published HCV-envelope sequences might be among the reasons for the poor response of HCV-4a to effective anti-HCV therapy against other genotypes of the virus.

Acknowledgment

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References


Figures And Tables

Fig. (1): Amplification of HCV-E2 fragments. Fig (1A) shows the PCR product following RT-PCR. A homogenous band of ~800 pb was detected at the expected M.wt. Fig (1B) shows the nested PCR amplification of smaller band of ~660 pb (lane 2) when the purified HCV-E2 fragment (lane 1) was used as template. Electrophoresis was carried out on 1% agarose gel and a 1kb DNA ladder was run on the same gel (lane L).

Fig. (2): Amplification products obtained from direct PCR on individual E. coli BL21 (DE3) colonies transformed with the pSC-A constructs into which the HCV-E2-encoding sequence was cloned using insert-specific primers. Specific bands of a M.wt. 800 bp were visualized in 4 out of 6 tested colonies reflecting high cloning efficiency. Electrophoresis was carried out on 1% agarose gel and a 1kb DNA ladder was run on the same gel (lane L).
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Fig. (3): Phylogenetic comparison of HCV-E2 sequence from Egyptian isolate with published ones with highest degree of homology.

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<tr>
<th>Description</th>
<th>Country</th>
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<th>Accession</th>
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Table (1) list of primers used in gene amplification and screening

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Table (2): Homology results of nucleotide sequence of our HCV-E2 gene to published HCV-envelope sequences in GenBank database

- Hepatitis C virus subtype 4a isolate 02C polyprotein gene, complete cds: USA 433 85% DQ418784 2007 [19]
- Hepatitis C virus subtype 4a isolate F7157 polyprotein gene, complete cds: USA 425 86% DQ418788 2007 [19]
- Hepatitis C virus subtype 4a isolate Egypt: 412 84% DQ988077 2007
- Hepatitis C virus subtype 4a isolate F753 polyprotein gene, complete cds: USA 411 84% DQ418787 2007 [19]
- Hepatitis C virus isolate 4a-J03P0185 envelope glycoprotein (env) gene, partial cds: USA 304 73% JQ064300 2011 [23]
- Hepatitis C virus subtype 1b isolate HCV-1b/US/BID-V156/2004, complete genome: USA 291 73% EU155226 2009
- Hepatitis C virus (2c) isolate 03_Cha_2002 polyprotein gene, partial cds: Argentina 285 72% JF511047 2011
- Hepatitis C virus (3h) isolate QC29, complete genome: Canada 277 70% JF735121 2013 [24]
- Hepatitis C virus (3h) isolate 811, complete genome: Somalia 277 70% JF735126 2013
- Hepatitis C virus (5a) isolate ZADGM0518 polyprotein gene, partial cds: South Africa 272 70% KC767832 2013
- Hepatitis C virus (6n) isolate WS142 polyprotein gene, partial cds: China 271 69% EU119974 2008 [25]
- Hepatitis C virus subtype 2b gene for polyprotein, complete cds, isolated from patient No.28: Japan 269 70% AB661432 2011
- Hepatitis C virus (6e) isolate 537798 polyprotein precursor, gene, complete cds: USA 263 69% EU408326 2009
- Hepatitis C virus type 5a (BE95) envelope protein (E1) gene, partial cds; envelope protein (E2) gene, complete cds: Belgium 258 69% L29578 1995 [26]